JOURNAL OF CLINICAL MICROBIOLOGY, June 2001, p. 2267–2271 Vol. 39, No. 6
0095-1137/01/$04.00+0 DOI: 10.1128/JCM.39.6.2267–2271.2001

Strategy for Diagnosis of Congenital Toxoplasmosis: Evaluation of Methods Comparing Mothers and Newborns and Standard Methods for Postnatal Detection of Immunoglobulin G, M, and A Antibodies

J. M. PINON,[1] H. DUMON,[2] C. CHEMLA,[1] J. FRANCK,[2] E. PETERSEN,[3] M. LEBECH,[4] J. ZUFFEREY,[5] M.-H. BESSIERES,[6] P. MARTY,[7] R. HOLLIMAN,[8] J. JOHNSON,[8] V. LUYASU,[9] B. LECOLIER,[10] E. GUY,[11] D. H. M. JOYNSON,[11] A. DECOSTER,[12] G. ENDERS,[13] H. PELLOUX,[14] AND E. CANDOLFI[15]*

Service de Parasitologie-Mycologie, CHU Hôpital Maison Blanche, UPRES EA 2070, IFR53, 51092, Reims,[1] Service de Parasitologie-Mycologie, Groupe Hospitalier de la Timone, 13385, Marseille,[2] Laboratoire de Parasitologie-Mycologie, CHU Rangueil, 31054, Toulouse,[6] Laboratoire de Parasitologie-Mycologie, Hôpital de l'Archet, 06202, Nice,[7] Laboratoires d'analyses de Biologie Médicale Levy, 75014 Paris,[10] Laboratoire de Microbiologie, CH Saint Philibert, 59462, Lomme,[12] Service de Parasitologie-Mycologie, CHU A. Michallon, 38043, Grenoble,[14] and Institut de Parasitologie et de Pathologie Tropicale, INSERM U 392, 67000, Strasbourg,[15] France; Staten Serum Institut 5 Artillerivej, 2300 Copenhagen,[3] and Department of Gynecology and Obstetrics, University Hospital Hvidovre, 2650 Copenhagen,[4] Denmark; Institut de Microbiologie, 1011 Lausanne, Switzerland[5]; Department of Medical Microbiology, St. George's Hospital, London,[8] and Public Health Laboratory, Singleton Hospital, Swansea SA2 80A,[11] United Kingdom; Clinique ParaUnivers Saint Pierre, 1340 Ottignies, Belgium[9]; and Institut für Virologie, Infektiologie und Epidemiologie, 70193 Stuttgart, Germany[13]

Received 15 December 2000/Returned for modification 1 February 2001/Accepted 2 April 2001

In a study involving 14 laboratories supported by the European Community Biomed 2 program, we evaluated immunologic methods for the postnatal diagnosis of congenital toxoplasmosis (CT). Among babies born to mothers who seroconverted to positivity for toxoplasmosis during pregnancy, we analyzed 55 babies with CT on the basis of persistent anti-*Toxoplasma* immunoglobulin G (IgG) at 1 year of life and 50 control babies without anti-*Toxoplasma* IgG at 1 year of life in the absence of curative treatment with pyrimethamine-sulfonamides. We tested in-house methods such as the enzyme-linked immunofiltration assay (ELIFA) or Immunoblotting (IB) for the detection of IgG or IgM; these methods allowed comparison of the immunologic profiles of the mothers and the infants. We compared ELIFA and IB with a commercial enzyme immunoassay (EIA) or in-house immunosorbent agglutination assay (ISAGA) for the detection of IgM or IgA. The performances of combinations of methods were also assessed. A cumulative sensitivity of 98% during a 1-year follow-up was obtained with the ELIFA plus ISAGA combination. Only one case of CT was missed by the ELIFA plus ISAGA combination, whereas three cases were missed by the IB plus ISAGA combination, even though 48% of patients with CT were treated with pyrimethamine-sulfonamides, which are known to inhibit antibody neosynthesis. A similar performance was obtained with either ELIFA or IB in combination with EIA. The difference in performance between ELIFA plus ISAGA and IB plus ISAGA was not statistically significant ($P = 0.31$), and we conclude that both combinations of tests can be used for the diagnosis of CT in newborns.

Toxoplasma gondii is a unicellular protozoan parasite. Although it is found worldwide, infection with the parasite is more prevalent in some regions in Europe and parts of the Caribbean and South America than in Asia, the United States, and Australia. *T. gondii* infection is prevalent throughout Europe, and the seroprevalence ranks from less than 20% in northern Europe to more than 60% in southern Europe (33). Seronegative women are at risk of infection, and as the infection is generally asymptomatic, it is difficult to diagnose. However, primary maternal *Toxoplasma* infection during pregnancy is frequently associated with transmission of *T. gondii* to the fetus (32). Fetal infection has unpredictable consequences, but sequelae may be prevented or reduced by early treatment (12, 30, 30a).

Postnatal diagnosis of congenital toxoplasmosis (CT) is crucial in two cases: (i) when clinical signs occur within the first 6 months of a child's life and no information on the mother's antenatal serostatus is available and (ii) when seroconversion is diagnosed during pregnancy, with or without antenatal diagnosis of CT. The first situation is mainly observed in countries where maternal screening is not performed (19). In countries where maternal screening is mandatory (France and Austria) or is done on a regular basis by obstetricians (Belgium, Switzerland, and Italy), suspected maternal toxoplasmosis calls for parasitologic and immunologic testing of the child at birth and during the first year of life (5).

Early postnatal diagnosis is necessary to identify infants qualifying for aggressive treatment based on pyrimethamine and sulfonamides (PS), which reduces the incidence of ocular sequel (23, 29, 31). However, CT is generally subclinical, es-

* Corresponding author. Mailing address: Institut de Parasitologie et de Pathologie Tropicale, INSERM U 392, 3 rue Koeberle, 67000, Strasbourg, France. Phone: 00 33 (0) 388 35 35 55. Fax: 00 33 (0) 388 36 42 02. E-mail: ermanno.candolfi@medecine.u-strasbg.fr.

pecially in countries with effective screening programs where treatment in utero reduces the risk of major complications (5, 12, 20, 29).

Parasitologic and immunologic means of diagnosis of CT are used during the first year of life. However, there are two major obstacles to postnatal diagnosis, namely, the poor sensitivity of *Toxoplasma* detection (7, 16) and the presence of maternal antibodies in the child, which hinders and delays the immunologic means of diagnosis. Standard methods for the detection of anti-*Toxoplasma* antibodies, such as enzyme immunoassay (EIA) and immunosorbent agglutination assay (ISAGA), fail to distinguish maternal antibodies, transmitted passively (immunoglobulin G [IgG]) or by leakage (IgM and IgA), and fetal or neonatal neosynthesized antibodies. Ten days after birth, only fetal IgM and IgA can be detected by these methods. Approaches based on the comparison of the immunologic profiles of the mother and the infant (referred to here as CIP methods), such as enzyme-linked immunofiltration assay (ELIFA) and immunoblotting (IB), which emerged in 1982 and 1985, respectively (27, 28), are claimed to distinguish maternal from fetal or neonatal neosynthesized antibodies. In the present collaborative study involving 14 laboratories supported by the European Community Biomed 2 program, we evaluated IB and ELIFA methods for the postnatal diagnosis of CT.

(Data from this study were presented by E. Petersen at the European Conference on Congenital Toxoplasmosis, Vienna, 29 June to 1 July 2000.)

MATERIALS AND METHODS

Patients. Patients were selected from 14 European centers on the basis of criteria established by the European Network on Congenital Toxoplasmosis (22).

CT. Fifty-five babies with CT were selected on the basis of the persistence of anti-*Toxoplasma* IgG at 1 year of life. The babies were born to mothers who had seroconverted to positivity for toxoplasmosis during pregnancy. We obtained 55 maternal serum samples collected at delivery or within the first month after delivery and 206 infant serum samples collected at birth or during the first year of life. Maternal seroconversion during pregnancy could not be precisely dated for 18% of the mothers. Among the remaining mothers, 4% seroconverted during the first trimester, 31% seroconverted during the second trimester, and 65% seroconverted during the third trimester. Seven mothers had symptomatic seroconversion (five had lymphadenopathy and two had fever). Treatment consisted of the PS combination in 47% of the mothers after detection of *Toxoplasma* DNA in amniotic fluid by PCR, 36% of the mothers received spiramycin, and 17% received no treatment. *Toxoplasma* was detected in the placenta or cord blood at birth in 10 cases. Of the 55 newborns infected with *Toxoplasma*, 9% had brain calcifications and a further 9% had retinochoroiditis.

Controls. Fifty case-control infants were selected on the basis of the absence of anti-*Toxoplasma* IgG after 1 year of life in the absence of curative PS treatment. Fifty maternal serum samples were obtained at delivery or within the first month of life, and 119 infant serum samples were obtained at birth or during the first year of life. None of the mothers had symptoms of toxoplasmosis during the pregnancy. Maternal treatment consisted of spiramycin in 44% of the cases, and one woman received the PS combination after detection of *Toxoplasma* DNA in amniotic fluid by PCR. The infant of the latter woman did not develop CT. No treatment was given to 46% of the mothers, and data were lacking for the remaining 8% of the mothers. The precise date of maternal seroconversion was not documented for 48% of the women; of the remaining women, 73% seroconverted during the first trimester, 23% seroconverted during the second trimester, and 4% seroconverted during the third trimester.

Equal volumes of serum were sent on dry ice to the laboratory in Reims, France, for ELIFA and ISAGA and to the laboratory in Marseille, France, for IB and EIA. The only pieces of information provided to these two laboratories were the date of birth, the date of sampling, and the maternal or neonatal nature of the sample. A data sheet with all clinical, therapeutic, and biological information was sent to the study coordinator in Strasbourg, France. Data and interpretations were checked during the first meeting, and the blinded files were opened in the presence of the coordinator and three others members of the network.

CIP methods. (i) ELIFA. ELIFA, which is based on the use of a microporous cellulose acetate membrane in a coimmunoelectrodiffusion procedure, simultaneously determines antibody specificity by immunoprecipitation and the antibody isotype by immunofiltration of an enzyme-labeled antibody through the membrane. The filtration step is conducted in a commercial ELIFA cell, which is controlled by an automatized peristaltic pump (26, 27). The profiles yield four parameters: the number of precipitating arcs; the isotype (IgG or IgM); the comparative specificities by the continuity (coalescence) of the arcs present in two serum samples, which proves that the same antigen is involved in the precipitate; and the relative amounts of antibodies with the same specificity in each sample. Fetal or newborn neosynthesized IgG and IgM antibodies are those present in neonatal serum and absent from maternal serum or those of a given specificity with a higher concentration in the neonatal sample than in the maternal sample.

(ii) IB. IB combines electrophoresis of toxoplasmic antigens under denaturing conditions, electrotransfer to a nitrocellulose membrane, and a specific antibody assay (similar to an EIA) that detects specific epitopes. The result is a band pattern that results from precipitation of the substrate on the membrane and that reveals the presence of the antigen-antibody complex (24). We used the IB procedure described by Remington et al. (28), with modifications (14). The presence of bands obtained with the infant's serum but not with the mother's serum reflects IgG or IgM antibody synthesis by the infant.

Standard methods for IgM and IgA detection. (i) IgM antibodies. Specific IgM was detected by using an EIA kit (EIA-M; Bio-Rad–Pasteur), according to the manufacturer's indications, and by an in-house ISAGA for IgM (ISAGA-M), which has been described elsewhere (26).

(ii) IgA antibodies. Specific IgA was detected by using an EIA kit (EIA-A; Bio-Rad–Pasteur), according to the manufacturer's indications, and an in-house ISAGA method for IgA (ISAGA-A) (26).

Statistical analysis. McNemar's nonparametric test was used for statistical analysis.

RESULTS

Sensitivity and specificity (Table 1) were calculated from the results for sera collected at three different points in the infants' lives: (i) at birth or within 10 days after birth, (ii) 0.5 to 1.5 months after birth, and (iii) 2 to 12 months after birth. Sensitivity and specificity were also expressed as cumulative results throughout the first year of life. These parameters were calculated for each method individually and in combination (17).

Comparative performances of immunologic tests in the neonatal period. EIA and ISAGA had specificities below 90%. ELIFA-G and IB-M had specificities of 100% and sensitivities of 64.2 and 56.7%, respectively. By ELIFA, the results for 5 serum samples from 42 infants with CT and 10 serum samples from 27 case-control infants were considered uninterpretable; the results for these infants were resolved with new samples collected within 10 days after birth.

When the results for two isotypes (for IgM and IgA by standard methods and for IgG and IgM by CIP methods) were combined, the sensitivity increased but the specificity remained low with the IgA-IgM combination. When the results of standard methods and CIP methods were combined, the sensitivities ranged from 81 to 92.1% but the specificities remained low (70.3 to 81.4%), owing to the poor specificities of the standard methods.

Comparative performances of immunologic tests at 0.5 to 1.5 months. Methods that detect a single isotype had sensitivities of no more than 73.3%. When the results for the detection of two isotypes by standard methods were combined, the sensitivity increased to 79.3%. When the results of the CIP methods and standard methods were combined, the sensitivities

TABLE 1. Sensitivities and specificities of individual and CIP methods for diagnosis of CT[a]

Parameter and method[b]	Performance at age:			Cumulative performance, 0–12 mo
	0–10 days	0.5–1.5 mo	2–12 mo	
Sensitivity				
Individual methods				
ISAGA-M	27/40 (67.5)[c]	17/27 (62.9)	12/42 (28.6)	43/53 (81.1)
ISAGA-A	29/40 (72.5)	18/27 (66.6)	21/42 (50)	44/53 (83)
EIA-M	26/42 (61.9)	17/29 (58.6)	6/42 (14.2)	35/54 (64.8)
EIA-A	30/42 (71.4)	17/30 (56.6)	16/42 (38)	45/54 (83.3)
ELIFA-G	26/38 (64.2 [5[d]])	22/30 (73.3)	38/43 (88.3)	48/54 (88.8)
ELIFA-M	7/42 (16.6)	6/29 (20.6)	6/40 (15)	12/54 (22.2)
IB-G	16/40 (40 [2])	19/30 (63.3)	29/43 (67.4)	36/55 (65.4)
IB-M	21/37 (56.7 [6])	20/29 (68.9)	23/41 (56)	37/54 (68.5)
Combined methods				
ISAGA-M + ISAGA-A	30/39 (76.9)	20/27 (74)	24/42 (57.1)	49/53 (92.4)
EIA-M + EIA-A	34/42 (80.9)	23/29 (79.3)	18/42 (42.8)	47/54 (87)
ELIFA-G + ELIFA-M	28/38 (73)	22/29 (75.8)	35/40 (87.5)	48/54 (88.8)
IB G + IB M	24/37 (64.8)	22/29 (75.8)	30/41 (73.1)	42/54 (77.7)
ELIFA-G + ELIFA-M + EIA-M + EIA-A	35/38 (92.1)	26/27 (96.2)	37/40 (92.5)	49/51 (96)
ELIFA-G + ELIFA-M + ISAGA-M + ISAGA-A	32/36 (88.8)	24/25 (96)	36/40 (90)	49/50 (98)
IB-G + IB-M + EIA-M + EIA-A	30/37 (81)	26/29 (89.6)	32/41 (78)	46/51 (90.1)
IB-G + IB-M + ISAGA-M + ISAGA-A	28/32 (87.5)	24/27 (88.8)	33/41 (80.4)	47/50 (94)
Specificity				
Individual methods				
ISAGA-M	21/27 (77.7)	27/28 (96.4)	34/34 (100)	43/50 (86)
ISAGA-A	21/27 (77.7)	29/29 (100)	35/35 (100)	44/50 (88)
EIA-M	24/27 (88.8)	32/32 (100)	37/37 (100)	47/50 (94)
EIA-A	23/27 (85.1)	31/32 (96.8)	37/37 (100)	43/50 (86)
ELIFA-G	17/17 (100 [10])	31/31 (100 [1])	37/37 (100)	50/50 (100)
ELIFA-M	27/27 (100)	31/31 (100)	33/33 (100)	50/50 (100)
IB-G	26/27 (96.2)	31/32 (96.8)	36/36 (100)	48/50 (96)
IB-M	27/27 (100)	31/31 (100 [1])	34/35 (97.1)	49/50 (98)
Combined methods				
ISAGA-M + ISAGA-A	20/27 (7)	27/28 (96.4)	34/34 (100)	46/50 (92)
EIA-M + EIA-A	21/27 (77.7)	31/32 (96.8)	37/37 (100)	41/50 (82)
ELIFA-G + ELIFA-M	17/17 (100 [10])	31/31 (100 [1])	33/33 (100)	50/50 (100)
IB-G + IB-M	26/27 (96.2)	30/31 (96.7)	34/35 (97.1)	48/50 (96)
ELIFA-G + ELIFA-M + EIA-M + EIA-A	20/27 (74)	27/28 (96.4)	34/34 (100)	42/50 (84)
ELIFA-G + ELIFA-M + ISAGA-M + ISAGA-A	21/27 (77.7)	30/31 (96.7)	37/37 (100)	41/50 (82)
IB-G + IB-M + EIA-M + EIA-A	19/27 (70.3)	26/28 (92.8)	33/34 (97)	40/50 (80)
IB-G + IB-M + ISAGA-M + ISAGA-A	22/27 (81.4)	29/31 (93.5)	34/35 (97.1)	38/50 (76)

[a] Sera from 55 babies with CT and 50 case-controls were separated according to the sampling date.
[b] ISAGA-M, ISAGA for IgM detection; ISAGA-A, ISAGA for IgA detection; EIA-M, IgM immunocapture-based EIA; EIA-A, IgA immunocapture-based EIA; ELIFA-G, ELIFA for IgG detection; ELIFA-M, ELIFA for IgM detection; IB-G, IB assay for IgG detection; IB-M, IB assay for IgM detection.
[c] Data represent number of positive serum samples/number of serum samples tested (percent).
[d] Data in brackets are the number of samples with uninterpretable ELIFA or IB assay results and are excluded from the final calculation.

ranged from 88.8 to 96.2%, while the specificities exceeded 92%.

Comparative performances of immunologic tests between 2 months and 1 year of life. The best performance was obtained when the results of the CIP methods were combined with those of the standard assays, with sensitivities ranging from 78 to 92.5% and with specificities exceeding 97%. The performances of the tests for the detection of anti-*Toxoplasma* IgM fell drastically, and sensitivities ranked only between 14.2 and 56%. On the other hand, the assays that detect anti-*Toxoplasma* IgA maintained reasonable performance, and when their results are combined with those of ELIFA or IB, the sensitivities are increased.

When we combined a CIP method with a standard method for detection of the three immunoglobulin isotypes, sensitivities exceeded 90% (98% sensitivity with ELIFA plus ISAGA, with 84% specificity). Only one case of CT was missed by the ELIFA plus ISAGA combination, whereas three cases were missed by the IB plus ISAGA combination. Similar performances were obtained when the CIP methods were individually combined with EIA instead of ISAGA. The difference between the best combination of these assays was, however, not statistically significant ($P = 0.31$).

DISCUSSION

At birth, the sensitivity of *Toxoplasma* detection is poor, ranging from 25 to 60.9% (7, 16). The use of immunologic methods is thus crucial for definitive postnatal diagnosis of CT. IgG is known to cross the placental barrier readily, but IgM

and IgA may "leak" through the placenta during labor and contaminate the newborn's blood. For instance, anti-*Toxoplasma* IgM and IgA antibodies were detected in this collaborative study in 12 and 22% of healthy neonates by EIA and ISAGA, respectively. New methods such as ELIFA and IB can distinguish maternal antibodies from fetal-neonatal neosynthesized antibodies and can be applied to samples collected at birth or during the first 10 days of life (4, 14, 26). When used together with standard methods for immunoglobulin detection, these new methods can improve the diagnosis of CT during the first month of life and later.

Detection of anti-*Toxoplasma* IgM is widely used for the diagnosis of CT. We compared an EIA with an ISAGA for IgM and, in our hands, found that the ISAGA for IgM performed better than the EIA for IgM, as reported previously (9, 15, 26). In-house and commercial EIAs are used to screen adult sera, in which the level of background noise due to natural IgM is high (21), and must be modified and carefully validated before being used with sera from infants (3, 3a). IB for IgM gave better results than ISAGA or EIA. This could be due to the better ability of IB to detect low levels of IgM. In general, the poor performances of assays that detect anti-*Toxoplasma* IgM is probably due to the fact that sampling is done at various times after infection and that IgM production may have ceased at birth.

The contribution of IgA detection to the early diagnosis of CT has been show in many studies (2, 6, 13, 25, 27). The methods used for IgA detection include ISAGA-A and EIA-A. However, recent reports on the comparative sensitivities of EIA-A and ISAGA-A are discordant (10, 13, 25, 26). In our hands, better results were achieved with ISAGA-A than with EIA-A. Overall, IgA detection was better than the IgM detection after 2 months of life unless we used IB for IgM. When the results for the two isotypes (IgM and IgA) were combined, Villena et al. (30) found that the sensitivity increased. These results were confirmed in our study and strongly support the use of an anti-*Toxoplasma* IgA detection test (ISAGA-A or EIA-A based on the major surface antigen protein of *T. gondii*, SAG1) in the panel of methods for serologic diagnosis of CT during the first months of life.

ELIFA methods detect specific antibodies present in neonatal serum and absent from maternal blood or detect a higher concentration of neonatal antibodies with the same specificity that they detect maternal antibodies. Pinon et al. (26) previously demonstrated that ELIFA had a sensitivity of 84.5% and a specificity of 99.9% during the first 3 months of life in infants with CT. We confirmed in our study that the sensitivity increased during the 1-year follow-up and reached a cumulative sensitivity of 88.8%. Specificity is high if we exclude the poor capacity of ELIFA to detect CT at birth, due to the hemoconcentration of cord blood, which inhibits the reading of the band pattern and renders the results uninterpretable. This problem can be overcome by testing a new sample collected 10 days later.

IB detects bands in the infant's serum which are absent from the mother's serum, pointing to antibody synthesis by the neonate. Franck and colleagues (14) were the first to demonstrate the usefulness of IB on a large scale. Chumpitazi et al. (4) achieved an overall sensitivity of 92.6% and a specificity of 89.1%. In our study, the sensitivity was lower and the specificity was higher. These differences could be due to the fact that IB kits have previously been homemade. Use of the semiautomatic computer-driven PHAST system from Pharmacia has increased the reproducibility of IB (4), and commercial IB kits are now available. Of note, as for ELIFA, the ability of these methods to detect neosynthesized antibodies allows increases in sensitivity during the 1-year follow-up.

Only one previous study compared ELIFA and IB methods for the detection of fetal and infant antibodies (4). In that study the IB method had a better resolution than the ELIFA method (4). On the contrary, in our hands, ELIFA had a better performance because it allowed the detection of higher concentrations of neosynthesized antibodies at birth, even when the target epitopes were identical to those of the maternal antibodies.

Combination of ELIFA methods with standard methods identified all but one of the CT cases during the first year of life in the present study. Analysis of the newborn whose CT was missed indicates that the newborn was correctly monitored. After a positive antenatal diagnosis by PCR with amniotic fluid, the mother received PS treatment during the third trimester. The child received the same treatment for 12 months after birth. The child's serology became negative at 6 months, and the child was still negative at 10 months. Immunologic markers of CT were detected only when treatment was stopped at the 14th month of life, leading to an immunologic rebound with a high level of IgG antibodies, as well as specific IgM and IgA. The other three false-negative results observed with the combination of the IB and standard methods involved similar patients treated with PS at an early stage of infection. In these individuals immunologic markers were detected only when treatment was withdrawn, owing to a rebound of toxoplasmic antibodies.

This suggests that PS treatment has an effect on serologic results. Previous investigations have suggested that the infant's IgG production was curbed by treatment, especially when parasite transmission occurred during labor (1). One study showed that treatment in utero could shorten the anti-*Toxoplasma* IgM-IgA response (26). Our data suggest that definitive postnatal serologic diagnosis can be delayed by treatment in utero. Therefore, confirmation of a diagnosis made in utero must be based on the persistence of anti-*Toxoplasma* IgG after 1 year of life and/or the detection of a serologic rebound when treatment is discontinued (8, 11, 18, 23).

No useful epidemiologic data on the prevalence of toxoplasmosis were obtained in the present study, as only individuals with proven cases were included. However, our findings demonstrate that early diagnosis of CT should be based on a combination of CIP methods with standard methods that detect fetal-neonatal neosynthesized IgG, IgM, and IgA antibodies. By this strategy, only one case of CT escaped detection, even though 48% of the individuals with CT were treated with PS, which is known to inhibit antibody neosynthesis. A prospective study in countries where prenatal screening is not done routinely is needed to confirm these conclusions.

ACKNOWLEDGMENTS

This work was supported by the European Research Network on Congenital Toxoplasmosis, the Biomed 2 program, and the Programme Hospitalier de Recherche Clinique du Ministère de la Santé of France.

We thank Philippe Thuilliez (Paris, France) for referring 17 CT patients, the technicians of the expert laboratories (Reims and Marseille, France) for assistance, and Eskerina Hoffmann (Strasbourg, France) for excellent secretarial skills.

REFERENCES

1. **Alford, C. A., Jr., S. Stagno, and D. W. Reynolds.** 1974. Congenital toxoplasmosis: clinical, laboratory, and therapeutic considerations, with special reference to subclinical disease. Bull. N. Y. Acad. Med. **50:**160–181.
2. **Bessières, M. H., C. Roques, A. Berrebi, V. Barrebi, V. Barre, M. Cazaux, and J. P. Seguela.** 1992. IgA antibody response during acquired and congenital toxoplasmosis. J. Clin. Pathol. **45:**605–608.
3. **Candolfi, E., and T. Kien.** 1988. Détection des IgM spécifiques dans le diagnostic de la toxoplasmose congénitale par une méthode d'immunopture ELISA sur billes. Détermination des paramètres d'utilisation. Rev. Fr. Lab. **178:**43–46.

3a. **Candolfi, E., M. H. Bessieres, P. Marty, B. Cimon, F. Gandilhon, H. Pelloux, and P. Thulliez.** 1993. Determination of a new cut-off value for the diagnosis of congenital toxoplasmosis by detection of specific IgM in an enzyme immunoassay. Eur. J. Clin. Microbiol. Infect.Dis. **12:**396–398.

4. **Chumpitazi, B. F., A. Boussaid, H. Pelloux, C. Racinet, M. Bost, and A. Goullier-Fleuret.** 1995. Diagnosis of congenital toxoplasmosis by immunoblotting and relationship with other methods. J. Clin. Microbiol. **33:**1479–1485.
5. **Daffos, F., F. Forestier, M. Capella-Pavlovsky, P. Thulliez, C. Aufrant, D. Valenti, and W. L. Cox.** 1988. Prenatal management of 746 pregnancies at risk for congenital toxoplasmosis. N. Engl. J. Med. **318:**271–275.
6. **Decoster, A., P. Gontier, E. Dehecq, J. L. Demory, and M. Duhamel.** 1995. Detection of anti-toxoplasma immunoglobulin A antibodies by Platelia-Toxo IgA directed against P30 and by IMx Toxo IgA for diagnosis of acquired and congenital toxoplasmosis. J. Clin. Microbiol. **33:**2206–2208.
7. **Desmonts, G., and J. Couvreur.** 1974. Isolation of the parasite in congenital toxoplasmosis: its practical and theoretical importance. Arch. Fr. Pediatr. **31:**157–166. (In French.)
8. **Djurkovic-Djakovic, O., S. Romand, R. Nobre, J. Couvreur, and P. Thulliez.** 2000. Serologic rebounds after one-year-long treatment for congenital toxoplasmosis. Pediatr. Infect. Dis. J. **19:**81–83.
9. **Duffy, K. T., P. J. Wharton, J. D. Johnson, L. New, and R. E. Holliman.** 1989. Assessment of immunoglobulin-M immunosorbent agglutination assay (ISAGA) for detecting toxoplasma specific IgM. J. Clin. Pathol. **42:**1291–1295.
10. **Faure, A. K., H. Fricker-Hidalgo, H. Pelloux, C. Bost-Bru, A. Goullier-Fleuret, and P. Ambroise-Thomas.** 1999. Lack of value of specific IgA detection in the postnatal diagnosis of congenital toxoplasmosis. J. Clin. Lab. Anal. **13:**27–30.
11. **Fortier, B., C. Coignard-Chatain, A. Dao, V. Rouland, A. S. Valat, D. Vinatier, and T. Lebrun.** 1997. Study of developing clinical outbreak and serological rebounds in children with congenital toxoplasmosis and follow-up during the first 2 years of life. Arch. Pediatr. **4:**940–976. (In French.)
12. **Foulon, W., I. Villena, B. Stray-Pedersen, A. Decoster, M. Lappalainen, J. M. Pinon, P. A. Jenum, K. Hedman, and A. Naessens.** 1999. Treatment of toxoplasmosis during pregnancy: a multicenter study of impact on fetal transmission and children's sequelae at age 1 year. Am. J. Obstet. Gynecol. **1802:**410–415.
13. **Foundrinier, F., C. Marx-Chemla, D. Aubert, A. Bonhomme, and J. M. Pinon.** 1995. Value of specific immunoglobulin A detection by two immunocapture assays in the diagnosis of toxoplasmosis. Eur. J. Clin. Microbiol. Infect. Dis. **14:**585–590.
14. **Franck, J., C. Mary, M. Laugier, H. Dumon, and M. Quilici.** 1992. Apport du Western blot au diagnostic de la toxoplasmose congénitale. Bull. Soc. Fr. Parasitol. **10:**3–7.
15. **Fricker-Hidalgo, H., H. Pelloux, C. Racinet, M. Bost, A. Goullier-Fleuret, and P. Ambroise-Thomas.** 1996. Congenital toxoplasmosis: specific IgM in fetal blood, cord blood and in the newborn. Ann. Biol. Clin. **54:**165–168.
16. **Fricker-Hidalgo, H., H. Pelloux, C. Racinet, I. Grefenstette, C. Bost-Bru, A. Goullier-Fleuret, and P. Ambroise-Thomas.** 1998. Detection of *Toxoplasma gondii* in 94 placentae from infected women by polymerase chain reaction, in vivo, and in vitro cultures. Placenta **19:**545–549.
17. **Gambini, D., R. Granier, and J. Ragguenau.** 1985. Méthodes d'évaluation des épreuves diagnostiques. Rev. Fr. Lab. **142:**21–34.
18. **Gratzl, R., M. Hayde, C. Kohlhauser, M. Hermon, G. Burda, W. Strobl, and A. Pollak.** 1998. Follow-up of infants with congenital toxoplasmosis detected by polymerase chain reaction analysis of amniotic fluid. Eur. J. Clin. Microbiol. Infect. Dis. **17:**853–858.
19. **Guerina, N. G.** 1994. Congenital infection with *Toxoplasma gondii*. Pediatr. Ann. **23:**138–142, 147–151.
20. **Hezard, N., C. Marx-Chemla, F. Foudrinier, I. Villena, C. Quereux, B. Leroux, D. Dupouy, M. Talmud, and J. M. Pinon.** 1997. Prenatal diagnosis of congenital toxoplasmosis in 261 pregnancies. Prenat. Diagn. **17:**1047–1054.
21. **Konishi, E.** 1991. Naturally occurring immunoglobulin M antibodies to *Toxoplasma gondii* in Japanese populations. Parasitology **102:**157–162.
22. **Lebech, M., D. H. Joynson, H. M. Seitz, P. Thulliez, R. E. Gilbert, G. N. Dutton, B. Ovlisen, and E. Petersen.** 1996. Classification system and case definitions of *Toxoplasma gondii* infection in immunocompetent pregnant women and their congenitally infected offspring. European Research Network on Congenital Toxoplasmosis. Eur. J. Clin. Microbiol. Infect. Dis. **15:**799–805.
23. **McAuley, J., K. M. Boyer, D. Patel, M. Mets, C. Swisher, N. Roizen, C. Wolters, L. Stein, M. Stein, W. Schey, et al.** 1994. Early and longitudinal evaluations of treated infants and children and untreated historical patients with congenital toxoplasmosis: the Chicago Collaborative Treatment Trial. Clin. Infect. Dis. **18:**38–72.
24. **Partanen, P., H. Turunen, R. Paasivuo, and P. Leinikii.** 1984. Immunoblot analysis of *Toxoplasma gondii* antigens by human immunoglobulin G, M, and A antibodies at different stages of infection. J. Clin. Microbiol. **20:**133–135.
25. **Patel, B., Y. Young, K. Duffy, R. P. Tanner, J. Johnson, and R. E. Holliman.** 1993. Immunoglobulin-A detection and the investigation of clinical toxoplasmosis. J. Med. Microbiol. **38:**286–292.
26. **Pinon, J. M., C. Chemla, I. Villena, F. Foudrinier, D. Aubert, D. Puygauthier-Toubas, B. Leroux, D. Dupouy, C. Quereux, M. Talmud, T. Trenque, G. Potron, M. Pluot, G. Remy, and A. Bonhomme.** 1996. Early neonatal diagnosis of congenital toxoplasmosis: value of comparative enzyme-linked immunofiltration assay immunological profiles and anti-*Toxoplasma gondii* immunoglobulin M (IgM) or IgA immunocapture and implications for postnatal therapeutic strategies. J. Clin. Microbiol. **34:**579–583.
27. **Pinon, J. M., and N. Gruson.** 1982. Interêt des profils immunologiques comparés E.L.I.F.A. dans le diagnostic précoce de la toxoplasmose congénitale. Lyon Med. **248:**27–30.
28. **Remington, J. S., F. G. Araujo, and G. Desmonts.** 1985. Recognition of different Toxoplasma antigens by IgM and IgG antibodies in mothers and their congenitally infected newborns. J. Infect. Dis. **152:**1020–1024.
29. **Roizen, N., C. N. Swisher, M. A. Stein, J. Hopkins, K. M. Boyer, E. Holfels, M. B. Mets, L. Stein, D. Patel, P. Meier, et al.** 1995. Neurologic and developmental outcome in treated congenital toxoplasmosis. Pediatrics **95:**11–20.
30. **Villena, I., D. Aubert, B. Leroux, D. Dupouy, M. Talmud, C. Chemla, T. Trenque, G. Schmit, C. Quereux, M. Guenounou, M. Pluot, A. Bonhomme, and J. M. Pinon.** 1998. Pyrimethamine-sulfadoxine treatment of congenital toxoplasmosis: follow-up of 78 cases between 1980 and 1997. Reims Toxoplasmosis Group. Scand. J. Infect. Dis. **30:**295–300.

30a. **Villena, I., C. Quereux, and J. M. Pinon.** 1998. Congenital toxoplasmosis: value of prenatal treatment with pyrimethamine-sulfadoxine combination. Prenat. Diagn. **18:**754–775.

31. **Wilson, C. B., J. S. Remington, S. Stagno, and D. W. Reynolds.** 1980. Development of adverse sequelae in children born with subclinical congenital Toxoplasma infection. Pediatrics **66:**767–774.
32. **Wong, S. Y., and J. S. Remington.** 1994. Toxoplasmosis in pregnancy. Clin. Infect. Dis. **18:**853–861.
33. **Zuber, P., and P. Jacquier.** 1995. Epidemiology of toxoplasmosis: worldwide status. Schweiz Med. Wochenschr. Suppl. **65:**19S–22S. (In French.)